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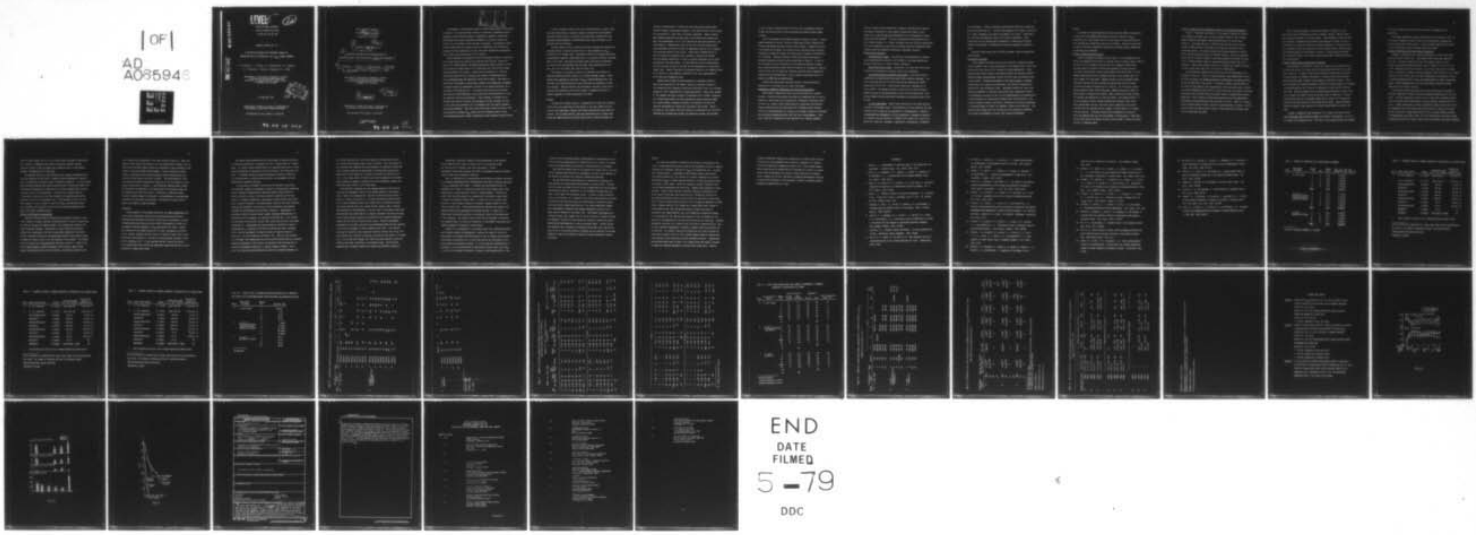

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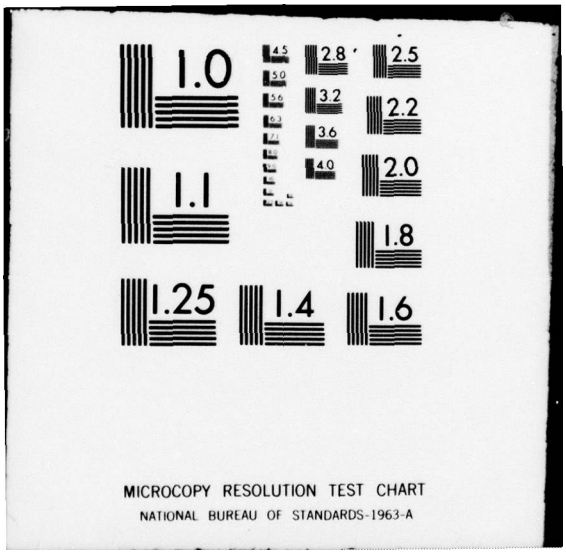
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TECHNICAL REPORT NO. 137

IS ANTIBIOTIC/STEROID POST-TREATMENT CAPABLE OF PREVENTING DEATH IN ESCHERICHIA COLI LD_{100} SHOCKED BABOONS?

L. B. Hinshaw, L. T. Archer, B. K. Beller-Todd, J. J. Coalson,

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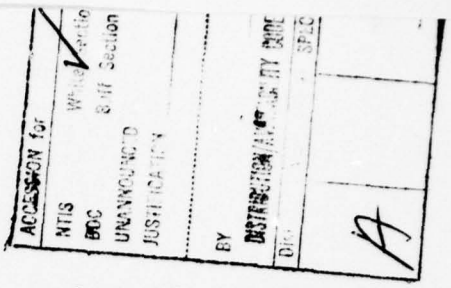

The response of the nonhuman primate to lethal Escherichia coli challenge is complex. The resulting shock involves a multitude of pathophysiological phenomena including marked changes in hemodynamic, metabolic, hematologic, microbiologic and tissue morphologic parameters, as shown by Coalson (6), Herman (11), and Hinshaw (15-18) and their colleagues. The underlying mechanisms of Escherichia coli shock are not well understood, and no effective treatment has been developed up to this time which reverses the physiopathologic effects and prevents death in this species.

Recent attempts to successfully treat the nonhuman primate in Escherichia coli induced shock with steroids only have met with failure. Herman and others (11) treated baboons with methylprednisolone sodium succinate following injection of lethal doses of live Escherichia coli and failed to demonstrate any benefit on survival. Recent work by Hinshaw et al. (18) and Coalson and others (6) has evaluated the effects of methylprednisolone on the pathophysiology and lethality of this form of shock. They found no significant differences in physiologic, metabolic and hematologic parameters in baboons receiving intermittent infusions of the steroid following low or high doses of Escherichia coli. The steroid used alone did not decrease the severity of any morphologic lesion produced by the Escherichia coli and the mortality rate was not decreased. These findings in primates are at variance with those of Pitcairn and colleagues (21) who increased survival in rats receiving Escherichia coli by treatment with the steroid dexamethasone sodium phosphate. They also differ from those of White and others (30) who successfully treated LD_{100} Escherichia coli endotoxin shock in dogs with methylprednisolone infused intermittently after endotoxin administration.

Clinical findings following the use of corticosteroids in septic shock have been inconclusive or negative; however, Schumer (27) has documented increased survival rates in patients with septic shock treated with dexamethasone sodium phosphate or methylprednisolone sodium succinate in conjunction with an antibiotic.

Current studies in this laboratory (19) have evaluated the separate and combined effects of steroid and antibiotic in dogs subjected to LD_{100} Escherichia coli induced shock: maintenance infusions of methylprednisolone and gentamicin together, administered after live organism infusion, prevented death in all dogs. Dogs receiving either agent alone were not protected and could not be distinguished from the group receiving Escherichia coli only, and 15 of 16 animals died within 30 hours (19).

The purpose of the present study was to determine the effects of a combined steroid-antibiotic regimen on a shocked nonhuman primate. Adult baboons were subjected to infusions of LD_{100} viable Escherichia coli, and treatment with methylprednisolone sodium succinate and gentamicin sulfate was evaluated. Results indicate that maintenance infusions of both corticosteroid and antibiotic are critical factors in promoting survival of baboons subjected to lethal Escherichia coli shock.

METHODS

Fourteen adult baboons (Papio c. cynocephalus) of either sex, captured in the wild, were stabilized for two months in the local animal facility. Prior to each experiment, baboons were fasted overnight and given water ad libitum. The following morning, they were restrained with a squeeze cage device and immobilized with ketamine hydrochloride, 14±0.5 milligrams per

kilogram intramuscularly. Animals were then administered sodium pentobarbital through a percutaneous catheter in the cephalic vein of the forearm and maintained at a light level of surgical anesthesia. Femoral vessels were exposed aseptically and cannulated in one hindlimb for pressure and heart rate measurements, fluid, live organism and drug administrations, and blood sampling. Baboons were placed on the left side with heating pads positioned above and below the body for temperature control, and a temperature probe was secured in the rectum. Animals were intubated orally in order to maintain an adequate airway passage, to provide for periodic positive pressure application in order to prevent atelectasis and to avoid regurgitation into the trachea. An equilibration period of approximately one hour was then utilized to provide for the development of a physiologic steady state. Animals received an intravenous infusion of 0.9 percent saline for 12 hours at a rate replacing insensible fluid loss, approximately 5 milliliters per kilogram per hour.

Baboons were divided into three groups as illustrated in Table I. Group A was comprised of five baboons receiving a two-hour infusion of live Escherichia coli prepared as previously described (1,15), at an average dosage of 2.1×10^{10} organisms per kilogram body weight. Group B was composed of five baboons receiving infusions of Escherichia coli (2.6×10^{10} organisms per kilogram body weight) and methylprednisolone sodium succinate (MPSS) (The Upjohn Company, Kalamazoo, Michigan) and gentamicin sulfate (GS) (Schering Pharmaceutical Corporation, Kenilworth, New Jersey). Group C consisted of four animals receiving infusions of Escherichia coli (3.0×10^{10} organisms per kilogram body weight) and gentamicin sulfate, but not MPSS.

In view of their relatively short half-lives (19), maintenance infusions of MPSS and GS were given at levels providing for optimal plasma concentrations.

Table II describes the treatment regimen of Groups A, B and C. Group B baboons received an initial infusion of MPSS after $0.7X10^{10}$ organisms per kilogram body weight had been infused (30-45 minutes). Gentamicin sulfate, 9 milligrams per kilogram, was infused during a one-hour period following completion of organism infusion (2-3 hours after initiation of Escherichia coli infusion). Periodic infusions of MPSS and GS were given during a constantly monitored 12-hour period as described in Table II. A total of 18 milligrams of GS per kilogram and 75 milligrams of MPSS per kilogram were infused during the 12-hour period followed by an intramuscular administration of GS, 4.5 milligrams per kilogram, and twice daily intramuscular injections of GS for three days. Group C animals received GS just as in Group B, but MPSS was not administered.

Animals were continuously monitored during a 12-hour period and observed for 7 to 28 days or until death intervened.

Hemodynamic, metabolic, hematologic and acid-base measurements

Mean arterial pressure and heart rate were monitored on a Sanborn recorder. Arterial blood samples were taken for determinations of insulin, hematocrit, white blood cell concentration, differential leukocyte concentrations, pH, lactate, serum glutamic-pyruvic transaminase and arginase. Glucose concentrations were determined with a Beckman glucose analyzer; insulin by radioimmunoassay and lactate by an enzymatic method on the DuPont ACA with glycine-hydrazine buffer, NAD^+ and lactic dehydrogenase. Total white blood cell concentrations were measured with a Coulter automatic

particle counter and the differential leukocyte distribution was made by microscopic examination of blood smears stained with Wright's stain. Values of pH, pCO_2 and pO_2 were determined with an Instrumentation Laboratories blood gas analyzer. Platelet concentrations were obtained with an MK-4 Platelet Counter (J. T. Baker Instrument Division, Milford, Connecticut) and fibrin degradation products were estimated using the Thrombo-Wellcotest procedure (Wellcome Research Laboratories, Beckenham, England).

Microbiologic procedures

Serum gentamicin levels. Concentrations were determined by bioassay using Staphylococcus epidermidic (ATCC 27626) as the assay organism and Antibiotic Medium No. 11 (Difco, Detroit, Michigan).

Baboon blood colony counts. Escherichia coli blood and serum concentrations were determined by standard colony count procedures.

Serum bacteriostatic and bactericidal levels. Levels were done by microdilution using Mueller-Hinton broth (Difco, Detroit, Michigan). The final volume per well was 0.1 milliliter and the inoculum was $1\text{-}4X10^5$ organisms per milliliter. Microdilution plates were incubated for 18 hours at 35°C. The highest dilution of serum with no visible bacterial growth was taken as the bacteriostatic level while the bactericidal level was the highest dilution which when inoculated on blood agar plates failed to grow..

In vitro experiments. Studies were carried out on the same organisms utilized in the present study. The Escherichia coli was tested by disc-agar diffusion (3) and found to be susceptible to chloramphenicol, furadantin, sulfamethoxazole-trimethoprim, colistin, gentamicin, tobramycin, kanamycin and amikacin, but was resistant to carbenicillin, ampicillin, ticarcillin, penicillin, oxacillin, vancomycin, cephalothin, tetracycline, clindamycin

and erythromycin. Minimal inhibitory concentrations (MICs) were determined by broth microdilution (7). The MICs were gentamicin (0.5 μg/ml), netilmicin (2.1 μg/ml), tobramycin (3.8 μg/ml) and amakacin (9.5 μg/ml). Escherichia coli was both inhibited and killed at 0.5 μg/ml of gentamicin. When Escherichia coli was tested against gentamicin in combination with MPSS, nembutal and ketamine, indifference was noted when compared to gentamicin alone.

Statistics were carried out utilizing a Student t test for paired and unpaired data.

Morphologic evaluation.

Tissue samples were taken from the left ventricle, intestine, adrenal glands, kidneys, liver, lungs and pancreas of the baboons within five to ten minutes after sacrifice with an overdose of sodium pentobarbital. These specimens were rapidly placed in phosphate buffered four percent formaldehyde-one percent glutaraldehyde fixative for both light and electron microscopic studies. Following procurement of the tissue samples, a thorough examination of all organs was accomplished, and additional samples were obtained for light microscopic study. Specimens obtained for light microscopy were embedded in Paraplast, and sections were subsequently stained with hematoxylin and eosin as well as with phosphotungstic acid-hematoxylin. Tissues obtained for ultrastructural studies were fixed overnight in the buffered aldehyde fixative, postfixed in Zetterqvist's fixative, dehydrated in ascending grades of alcohol and embedded in Epon 812 and Araldite. Thin sections were stained with lead citrate and uranyl acetate and examined with either an RCA-EMU-3G or Hitachi HS-9 electron microscopes.

RESULTS

The effects of methylprednisolone sodium succinate (MPSS) and gentamicin sulfate (GS) in the baboon administered a two-hour infusion of LD_{100} Escherichia coli were studied during a 12-hour period of continuous monitoring, followed by an observation period up to 28 days. Results are described in terms of survival, hemodynamics, metabolism, acid-base status, hematology, microbiology and morphology.

Effects of MPSS and GS on survival

Table III presents the baboon survival data. All five baboons administered live organisms alone died in an average time of 19 hours (range, three to 42 hours); all five baboons receiving the full treatment of MPSS and GS following onset of organism infusion were permanent survivors and were arbitrarily sacrificed at various times between seven and 28 days; all four baboons receiving only GS as treatment were dead in an average time of 24 hours (range, 15 to 35 hours). Baboons of Group B (fully treated) showed remarkable recovery and were drinking and moving about within 24 hours following the onset of Escherichia coli infusion. All five fully treated baboons appeared normal within three days, demonstrating normal behavior, eating habits, alertness and strength. Bowel function was normal (absence of diarrhea) and production of urine was evident. Non-treated animals, if surviving beyond 12 hours, became progressively lethargic, were anuric, often demonstrated diarrhea, and while usually remaining in an upright position with the forearms used for support, quietly expired in a slumped vertical position. Untreated animals were non-aggressive and passive, while the opposite was true with the treated, surviving group. These observations were regularly observed and were quite reliable in terms of predictability of impending death.

Effects of MPSS and GS on hemodynamic, metabolic and acid-base parameters

Figure 1 illustrates the effects of live organism infusion on arterial blood pressure and heart rate in control baboons (Group A), fully treated baboons (Group B) and baboons receiving Escherichia coli and GS alone (Group C). The Escherichia coli infusion period and the various infusion periods of MPSS and GS are displayed on the abscissa during the 12-hour observation period. All animals became significantly hypotensive ($p<0.05$) within two hours after the onset of organism infusion. While the subsequent period (two to 12 hours) revealed a sustained hypotensive response in Groups A and C, treated animals (Group B) maintained relatively normal mean aortic pressures. Heart rates increased notably in all groups, but tended to subside to lower values after eight hours in the fully treated group.

The relationships between serum glucose and insulin concentrations are displayed in Table IV. Group A, untreated baboons, were hyperglycemic during the first two hours, but became progressively hypoglycemic and hypoinsulinemic during the remainder of the period. In contrast, animals receiving MPSS and GS (Group B) were generally more hyperglycemic during the early phase of shock, but during subsequent periods (four to 12 hours and seven to 28 days) maintained glucose and insulin levels within the normal range or increased above control. Baboons No. 9 and 12 (Group C), receiving organisms and GS only, demonstrated hypoglycemia and hypoinsulinemia and died within 16 hours. Normal or elevated insulin concentrations followed a period of hypoinsulinemia in animals 3 and 15 up to 24 hours, and these animals died at a later time (29 to 42 hours) when insulin values were not known.

Table V displays changes in acid-base parameters in baboons at four, eight and 12 hours following the onset of organism infusion. Mean pH values were relatively constant in all three groups. Mean decreases in pCO_2 and HCO_3^- and increases in lactate and base deficit were observed in Groups A, B and C. Distortion of the data at 12 hours was caused by Baboon No. 11 (Group B) which had a temporary blood clot obstruction of the tracheal cannula, elevating pCO_2 and decreasing pO_2 to 27 millimeters of mercury. Values of pO_2 (not shown) were relatively similar in all groups, demonstrating very little change during the 12-hour period.

Effects of MPSS and GS on hematologic parameters

Mean decreases in neutrophil, total white blood cell and platelet concentrations were seen in all three groups following administration of live organisms (Figure 2). Of special note was the observation that white blood cells, including mature and immature neutrophils, in Group B animals were elevated above the values seen in Group A and C baboons at eight and 12 hours. There were essentially no immature neutrophil elevations in either Group A or C animals following Escherichia coli infusion in comparison to their control values at zero time. Surviving baboons (Group B) demonstrated recoveries of white blood cells to base-line values at seven to 28 days (time of sacrifice). Platelet concentrations were not influenced by MPSS and GS, values in all groups progressively falling during the 12-hour observation period. Surviving baboons (Group B) showed a large overshoot in platelet concentration at seven to 28 days.

Changes in hematocrit were negligible in all groups and Table VI shows that the average rate of saline infusion was similar in the groups, 4.3 to 5.3 milligrams per kilogram per hour. The rate of fluid administration was adjusted

to replace insensible fluid loss and was reduced if hematocrits fell excessively.

Fibrinogen degradation product concentrations are displayed in Table VII and similar increases are seen in each of the three groups, progressively occurring during the 12-hour period. Values returned essentially to control in surviving baboons (Group B) between seven and 28 days.

Effects of MPSS and GS on microbiological parameters

Changes in Escherichia coli blood concentrations in each of the three groups are shown in Table VIII. Each baboon blood culture was negative at zero time. Following completion of the live organism infusion at 120 minutes, concentrations were essentially equal in Groups A, B and C (1.8 to $2.1X10^7$ organisms per milliliter blood). By 12 hours, mean values in Groups B and C, including all baboons receiving gentamicin, were similar (2.3 to $2.9X10^2$ organisms per milliliter), while the mean concentration of organisms in Group A, receiving Escherichia coli alone, were approximately an order of magnitude higher ($1.6X10^3$ organisms per milliliter) than those in Group B ($2.3X10^2$ organisms per milliliter) ($p<0.05$).

Serum bacteriostatic and bactericidal values associated with gentamicin infusions summarized in Table IX confirm the marked effectiveness of the antibiotic. Dilution procedures indicate a ten- to forty-fold increased bacteriostatic and bactericidal effectiveness in Group B baboons receiving both MPSS and GS. Higher values seen in animals receiving GS alone at eight and 12 hours are presumably due to the higher plasma levels of GS, as shown at the lower portion of Table IX. Serum concentrations of GS in Group B baboons averaged 17 micrograms per milliliter (range, 10 to 21) from three to five hours; eight micrograms per milliliter (range, three to 10) at 8.5 hours, and eight micrograms

per milliliter (range, six to 11) at 12 hours after the onset of Escherichia coli infusion. Subsequent values were not measured; however, animals received a total of nine milligrams per kilogram per day, in two separate dosages, intramuscularly for three days.

The question arose as to why Escherichia coli organism concentrations in the blood remained at high levels in baboons receiving gentamicin (Groups B and C) in comparison to animals given no antibiotic (Group A). In vitro studies were therefore conducted with baboon blood and serum to which were added live organisms and combinations of MPSS and GS in similar concentrations as were utilized in the in vivo experiments. Findings from a single study are illustrated in Figure 3 and show that organisms are completely and quickly destroyed in vitro in the presence of GS. At 60 minutes in vitro, all are dead; whereas in vivo, as shown in Table VIII above, concentrations equal to or exceeding 10^2 organisms per milliliter of blood are observed by the end of the 12-hour treatment period.

Effects of MPSS and GS on morphology

Autopsies were performed and specimens were obtained from heart, lungs, liver, adrenals, kidneys, pancreas and gastrointestinal tract for examination by light and electron microscopy; however, the present paper presents only light microscopy findings. Examination of tissue samples was conducted without prior knowledge of the kind of experiment conducted on the animal. The most prominent finding was the hemorrhage observed in the adrenal glands of baboons in Groups A and C, with the exception of two animals. The adrenal lesion in Group A was indistinguishable from that in Group C. Three of the fully treated baboons, euthanized between 15 and 28 days, showed normal adrenal architecture while two of the animals, sacrificed at seven to nine

days, revealed mild congestion of the zona fasiculata (Group B). Renal and hepatic fibrin thrombi were absent in all but three animals (Groups A and C). There were no serious tubular lesions as evidenced by light microscopy in the kidneys of the GS and MPSS treated baboons. Similar nonspecific tubular lesions, including cellular edema and hyaline droplet accumulation, were observed in both Group A and Group C animals. The gastrointestinal tract was essentially normal, showing only ectasia of villus tip vessels in three baboons (two animals in Group B). Lung morphology revealed normal numbers of neutrophils and monocytes in the capillaries of all animals except the two baboons dying within five hours. Alterations in these two animals included increased numbers of neutrophils, microatelectasis, and hemorrhage consistent with the shock lung syndrome. The pancreatic tissue from all baboons was normal by light microscopy.

DISCUSSION

The key question in the present study was, *"Is lethal Escherichia coli induced shock effectively treated with corticosteroid and antibiotic?"* Experiments were designed to evaluate the efficacy of maintenance infusions of methylprednisolone sodium succinate (MPSS) and gentamicin sulfate (GS) in nonhuman primates subjected to LD_{100} Escherichia coli shock. Studies were conducted on the baboon because of its closer phylogenetic proximity to man, thereby extending the application of findings to human septic shock. Experiments were designed as a follow-up to those recently reported in dogs by this laboratory (19). It was expected that well controlled studies in a primate model would clarify the controversy concerning the use of corticosteroids in human septic shock.

The present study demonstrated the effectiveness of combined infusions of steroid and antibiotic in promoting survival in Escherichia coli induced shock. All control animals administered live organisms alone (N=5) or those receiving organisms in conjunction with the antibiotic (N=4) died within 42 hours. All baboons receiving combined maintenance infusions of MPSS and GS, following onset of organism administration, were permanent survivors, demonstrating remarkable recovery.

Is the success of treatment with steroid and antibiotic associated with favorable changes in hemodynamic, metabolic and hematologic parameters? Several important differences were observed between treated animals of Group B, controls of Group A and those receiving organisms and GS alone (Group C). Systemic hypotension, hypoglycemia, hypoinsulinemia and neutropenia, regularly observed in untreated baboons (Group A) and those receiving organisms and gentamicin alone (Group C), were prevented by the combined treatment of corticosteroid and antibiotic (Group B). Hypoglycemia is a consistent finding in several animal species following administration of Escherichia coli or endotoxin as reported by Griffiths et al. (9), Hinshaw and associates (14-16,18,19), Schuler (26), White (30) and their colleagues, and Archer (1). Hypoglycemia has been ascribed to inhibition of hepatic gluconeogensis, as reported by Griffiths and Groves and their colleagues (9,10), and the development of hepatic lesions as found by Archer (1), Balis et al. (2) and Hinshaw and co-workers (14). Berry (4) and Holtzman and colleagues (20) demonstrated that corticosteroid administration stimulated hepatic gluconeogensis, while Sculer and associates (26) observed that it supported carbohydrate metabolism in shocked nonhuman primates. Galvin et al. (8) found that methylprednisolone stabilizes lysosomal membranes in

the isolated perfused cat liver after endotoxin administration, and by this action should support normal hepatic function. Balis and others (2) also reported that treatment with corticosteroid in endotoxin shocked non-human primates inhibited the development of disseminated intravascular coagulation, fibrin deposition in hepatic sinusoids and lesions of the liver. This latter action of steroid could not be assayed in the present study inasmuch as changes in platelet and fibrin degradation product concentrations were similar in all three groups.

Corticosteroid treatment may have enhanced metabolic activities by improving the hemodynamic status of the shocked baboons of the present study. Sambhi and colleagues (24) reported increased cardiac outputs in patients in shock; Hinshaw et al. (13) observed elevated coronary blood flow in canine endotoxin shock; while Vaughn (29) and Hinshaw and others (12) found enhanced blood flow through several regional beds, following administration of corticosteroids. The fact that in the present study fluid administration was used primarily to replace insensible loss and pH and pO_2 values remained normal supports the probability that adequate hemodynamic support was the result of steroid action alone. Effective hemodynamic improvement of the renal circulation by methylprednisolone has been reported by Sullivan and Cavanagh in canine endotoxin shock (28). Since baboons subjected to endotoxin shock demonstrate markedly lowered renal blood flows and elevated renal vascular resistances, according to the studies of Cavanagh et al. (5) and Rao and Cavanagh (22), MPSS could have exerted a beneficial effect on the renal circulation in the present study. Urine flow was observed only in baboons treated with steroid and antibiotic (Group B).

Morphologic evaluation revealed striking hemorrhage in the adrenals after Escherichia coli shock in Groups A and C in the present study, but this was not a finding in the surviving animals. Similar hemorrhagic lesions were found by Rich (23) in the adrenal cortex of patients dying of shock due to bacterial infection.

Why is a combination of corticosteroid and antibiotic treatment successful in preventing pathophysiological manifestations and death in baboons subjected to LD_{100} Escherichia coli shock? It appears that methylprednisolone and gentamicin are linked in a protective association since neither agent alone demonstrated a single beneficial effect in shocked baboons. Schloerb and others (25) found that bacteria are extensively distributed in many organs in canine live organism induced shock. In order to kill or inhibit these bacteria, it would appear necessary to transport adequate amounts of antibiotic to these sites. Since methylprednisolone improves both systemic and regional blood flow by peripheral and cardiac actions, it should aid in effectively distributing gentamicin to peripheral sites for the destruction of the organisms. The notable effect of MPSS and GS treatment in elevating the concentrations of circulating neutrophils in the present study would also augment phagocytic antibacterial effectiveness.

Because of its augmentation of peripheral blood flow, methylprednisolone should enhance hepatic gluconeogenesis, increase the supply of insulin by the pancreas, and promote acid-base balance as well as improve the peripheral distribution of antibiotic, all of which may explain the advantages of combined steroid and antibiotic therapy. It is not known why concentrations of organisms in vivo were maintained at significantly higher levels than those in vitro in the presence of gentamicin; however, serum concentrations of the

antibiotic were considered adequate (approximately 10 micrograms per milliliter) and blood concentrations of Escherichia coli at 12 hours in treated animals were approximately ten percent of those receiving no treatment.

Data from the present study do not support the "shock lung" or the "gut lesion" concepts pertaining to the pathogenesis of septic shock because of the absence of pulmonary and intestinal morphologic pathology.

Why has there been a wide disparity of observations concerning the effectiveness of corticosteroid administration in septic shock? Findings from this study and others (6,18,19) clearly demonstrate that corticosteroid administration alone is ineffective in the treatment of Escherichia coli shock in both dogs (19) and baboons (6,18). In order to obtain effective action of the steroid, it is essential that steroid and antibiotic are given concomitantly and that both agents are administered in appropriate doses to assure optimal plasma concentrations. Our recent experiences suggest that large doses of steroid do not depress phagocytic activity (17) or delay time of recovery of the animal (19). The present investigation and a previous report (19) suggest that an initial infusion of steroid followed by subsequent infusions of both steroid and antibiotic reverse the pathophysiological manifestations of shock and prevent death. Further studies are planned by this laboratory to determine the maximal delay time during shock prior to instituting effective treatment and to ascertain the minimum dosages of steroid and antibiotic required to assure successful recovery from shock.

SUMMARY

This study was designed to determine the efficacy of maintenance infusions of methylprednisolone sodium succinate and gentamicin sulfate in baboons subjected to intravenous infusions of LD_{100} live Escherichia coli. Fourteen adult baboons (Papio c. cynocephalus) were fasted, lightly anesthetized, instrumented aseptically, and infused with $2.4X10^{10}$ organisms per kilogram body weight (LD_{100}). Animals were monitored for 12 hours and observed seven to 28 days or until death. Saline was infused to replace insensible fluid loss and body temperatures were controlled. Baboons were subjected to three regimens: Group A (N=5) received a two-hour infusion of Escherichia coli alone; Group B (N=5) was given Escherichia coli followed by infusions of both methylprednisolone sodium succinate (MPSS) and gentamicin sulfate (GS); Group C (N=4) was administered Escherichia coli followed by gentamicin sulfate. Group B baboons received 75 milligrams per kilogram MPSS slowly infused in divided doses at 30, 150, 360 and 600 minutes following the onset of Escherichia coli administration, and 18 milligrams per kilogram GS infused in divided doses at 130, 360 and 540 minutes with four milligrams per kilogram given intramuscularly at 12 hours and twice daily for three days. Systemic hypotension occurred in all animals within two hours of Escherichia coli infusion but pressures subsequently increased in baboons receiving both MPSS and GS (Group B). All animals infused with Escherichia coli with or without GS died within 42 hours, while all fully treated baboons (Group B) demonstrated rapid recovery within three days and were permanent survivors. Hypoglycemia and hypoinsulinemia were not seen in the treated group and notable increases in mature and immature neutrophils occurred within eight hours. Adverse

pulmonary morphologic changes were observed only in animals dying acutely, although pH and pO_2 parameters were relatively unchanged in all baboons. Significant morphologic changes were absent in all fully treated baboons. Blood concentrations of Escherichia coli organisms at 12 hours in treated animals were approximately ten percent of those receiving no treatment. Results suggest the critical importance of combining maintenance infusions of steroid and antibiotic in the prevention of death in nonhuman primates subjected to Escherichia coli shock.

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TABLE I. DOSAGES OF ESCHERICHIA COLI ADMINISTERED TO BABOONS

Group	Description of group	Baboon No.	Sex	Weight, kgm.	No. of E. coli org. infused per kgm. body wt.*
A	E. coli alone	1	F	15.8	$2.1X10^{10}$
		4	F	16.2	$2.1X10^{10}$
		7	M	19.9	$1.7X10^{10}$
		10	M	19.0	$2.5x10^{10}$
		13	M	18.2	$2.3X10^{10}$
		Mean		17.8	$2.1X10^{10}$
B	E. coli + methylprednisolone sodium succinate + gentamicin sulfate	2	F	16.0	$2.1X10^{10}$
		6	F	13.0	$2.1X10^{10}$
		8	M	14.5	$2.0X10^{10}$
		11	M	22.5	$4.2X10^{10}$
		14	M	18.3	$2.5X10^{10}$
		Mean		16.9	$2.6X10^{10}$
C	E. coli + gentamicin sulfate	3	F	17.8	$2.1X10^{10}$
		9	M	18.7	$1.9X10^{10}$
		12	M	18.5	$4.6X10^{10}$
		15	M	12.1	$3.2X10^{10}$
		Mean		16.8	$3.0X10^{10}$

*volume of organisms infused = 2.1 ml/kg.

TABLE II. TREATMENT REGIMEN IN BABOONS SUBJECTED TO ESCHERICHIA COLI-INDUCED SHOCK

Group	Agent administered	Dosage	Time after onset of E. coli infusion	Duration and route of administration
A	E. coli organisms	2.1 ml/kg*	Zero time (0)	0-120 min, IV
B	E. coli organisms	2.1 ml/kg	Zero time (0)	0-120 min, IV
	Methylprednisolone†	30 mg/kg	+30 min	15 min, IV
	Gentamicin§	9 mg/kg	+130 min	60 min, IV
	Methylprednisolone	15 mg/kg	+150 min	120 min, IV
	Gentamicin	4.5 mg/kg	+365 min	30 min, IV
	Methylprednisolone	15 mg/kg	+365 min	120 min, IV
	Gentamicin	4.5 mg/kg	+9 hr	30 min, IV
	Methylprednisolone	15 mg/kg	+10 hr	120 min, IV
	Gentamicin	4.5 mg/kg	+12 hr	IM
	Gentamicin	4.5 mg/kg	Twice daily, 3 days	IM
C	Same procedure as group B, but no methylprednisolone administered			

Saline infusions are substituted for drugs, when latter are not administered.

*See Table I for number of organisms per kgm in individual animals.

†Methylprednisolone sodium succinate.

§Gentamicin sulfate.

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Group	Agent administered	Dosage	Time after onset of E. coli infusion	Duration and route of administration
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	Gentamicin§	9 mg/kg	+130 min	60 min, IV
	Methylprednisolone	15 mg/kg	+150 min	120 min, IV
	Gentamicin	4.5 mg/kg	+365 min	30 min, IV
	Methylprednisolone	15 mg/kg	+365 min	120 min, IV
	Gentamicin	4.5 mg/kg	+9 hr	30 min, IV
	Methylprednisolone	15 mg/kg	+10 hr	120 min, IV
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Saline infusions are substituted for drugs, when latter are not administered.

*See Table I for number of organisms per kgm in individual animals.

†Methylprednisolone sodium succinate.

§Gentamicin sulfate.

TABLE III. SURVIVAL DATA IN BABOONS RECEIVING ESCHERICHIA COLI ORGANISMS AND TREATED WITH METHYLPREDNISOLONE SODIUM SUCCINATE AND GENTAMICIN SULFATE

Group	Description of group	Baboon No.	Survival time
A	E. coli alone	1	10.5 hr
		4	3 hr
		7	5 hr
		10	32 hr
		13	42 hr
B	E. coli + methylprednisolone sodium succinate + gentamicin sulfate	2	7 days*
		6	9 days*
		8	15 days*
		11	28 days*
		14	16 days*
C	E. coli + gentamicin sulfate	3	29 hr
		9	17 hr
		12	15 hr
		15	35 hr

*Euthanized.

TABLE IV. RELATIONSHIP BETWEEN GLUCOSE AND INSULIN IN BABOONS ADMINISTERED INFUSIONS OF ESCHERICHIA COLI ORGANISMS

Group	Description of group	Baboon No.	Parameter	Control	Hrs 2	3	4	5	6	8	10	12-16	24	29-42	Days 7-28
A	E. coli alone	1	Glucose*	90	105		55	41	43	49	37[d]				
			Insulin†	80			23		1		0				
		4	Glucose	96	109	56[d§]									
			Insulin	47	26	0									
		7	Glucose	73	119		26	21[d]							
			Insulin	18			8								
		10	Glucose	86	133		99	72	68	70	76	92		*d*	
			Insulin	44						1		17			
		13	Glucose	67	58		30	25	22	27	32	41		*d*	
			Insulin	30	2					4		6			
B	E. coli + methylprednisolone + gentamicin	2	Glucose	84	145		94		88	100	108	110			83
			Insulin	56			18				24	35	34		34
		6	Glucose	92	101		72		60	70	106	104			87
			Insulin	60			20				22	22			--
		8	Glucose	77	85		84		80	84	94	98			126
			Insulin	14			10			12		54			160
		11	Glucose	160	164		211			110	89	168			83
			Insulin	265			189			163		208			220
		14	Glucose	71	135		55		52	70	71	73			--
			Insulin	76			21			26		59			240

				Control	2	3	4	5	6	8	10	12-16	24	29-42
C	E. coli + gentamicin	3	Glucose	102	165		115		82	78	76	69	68	*d*
			Insulin	60			78				14	53	90	
		9	Glucose	77	74		33		31	33	32	44[d]		
			Insulin	32			2			3		1		
		12	Glucose	77	70		63		64	64	67	68[d]		
			Insulin	13			5			2		4		
		15	Glucose	62	87		57		47	51	56	72		*d*
			Insulin	13			7			4		71		

*glucose, mg/dl

†insulin, uU/ml

§*d* = death

TABLE V. CHANGES IN ACID-BASE PARAMETERS IN BABOONS SUBJECTED TO ESCHERICHIA COLI SHOCK AND TREATED WITH STEROID AND ANTIBIOTIC

GROUP A--E. coli alone						GROUP B--E. coli + methylprednisolone + gentamicin						GROUP C--E. coli + gentamicin					
Baboon No.	pH	pCO_2	HCO_3^-, mEq/L	Base excess, mEq/L	Lactate	Baboon No.	pH	pCO_2	HCO_3^-, mEq/L	Base excess, mEq/L	Lactate	Baboon No.	pH	pCO_2	HCO_3^-, mEq/L	Base excess, mEq/L	Lacta
Zero time																	
1	7.34	44	23	-3	4.1	2	7.42	38	24	+1	1.8	3	7.42	40	25	+1	12.6
4	7.34	40	21	-4	8.9	6	7.29	40	18	-8	10.7	9	7.38	42	24	-1	6.2
7	7.41	43	26	+2	8.0	8	7.33	40	20	-5	9.8	12	7.34	43	22	-3	8.9
10	7.33	53	27	-1	7.1	11	7.30	43	20	-6	38.3	15	7.33	47	24	-3	9.8
13	7.39	47	28	+2	6.7	14	7.39	44	26	+1	2.7						
Mean	7.36	45	25	-0.8	7.0	Mean	7.35	41	22	-3.4	12.7	Mean	7.37	43	24	-1.5	9.4
SE	0.02	2	1	1.2	0.8	SE	0.03	1	2	2.0	6.7	SE	0.02	2	1	1.0	1.3
+4 hours																	
1	7.38	23	13	-8	54.0	2	7.43	21	13	-6	44.1	3	7.45	27	18	-2	63.0
4	--	--	--	--	--	6	7.34	33	17	-7	27.6	9	7.41	25	15	-5	50.7
7	7.40	30	18	-4	57.0	8	7.44	30	20	-1	24.0	12	7.37	31	17	-5	44.5
10	7.40	34	20	-2	34.7	11	7.30	35	16	-9	73.0	15	7.37	32	18	-5	32.0
13	7.40	36	21	-1	28.1	14	7.49	32	24	+3	28.5						
Mean	7.40	31	18	-3.8	43.5	Mean	7.40	30	18	-4.0	39.4	Mean	7.40	29	17	-4.3	47.6
SE	0.01	3	2	1.5	7.1	SE	0.03	2	2	2.2	9.1	SE	0.02	2	1	0.8	6.5
p*		.01				p*		.005			.01	p*	.001	.005	.005	.02	.01

						+8 hours										
1	7.39	22	13	-8	104.9	2	7.49	18	13	-4	41.4	3	7.44	22	14	-5	85.5
4	--	--	--	--	--	6	7.34	30	15	-8	50.7	9	7.39	23	13	-7	79.2
7	--	--	--	--	--	8	7.47	25	18	-2	27.6	12	7.30	34	16	-9	71.2
10	7.36	29	16	-7	49.8	11	7.34	35	18	-6	81.0	15	7.37	32	18	-5	40.0
13	7.41	31	19	-3	41.8	14	7.39	30	18	-4	35.6						
Mean	7.39	27	16	-6.0	65.5	Mean	7.41	28	16	-4.8	47.3	Mean	7.38	28	15	-6.5	69.0
SE	1.45	3	2	1.5	19.8	SE	0.03	3	1	1.0	9.2	SE	0.03	3	1	1.0	10.1
p*		.02				p*		.005	.05		.005	p*		.01	.01	.02	.01
						+12 hours										
1	--	--	--	--	--	2	7.43	22	14	-5	36.5	3	7.49	17	13	-5	99.9
4	--	--	--	--	--	6	7.37	24	13	-8	50.7	9	7.34	23	12	-11	73.9
7	--	--	--	--	--	8	7.43	29	19	-2	32.0	12	7.25	33	14	-13	89.9
10	7.32	30	15	-9	66.8	11	6.90	84	15	-17	140.6	15	7.30	31	15	-10	54.3
13	7.39	25	15	-6	44.1	14	7.48	31	23	-1	44.5						
Mean	7.36	28		-7.5	55.5	Mean	7.32	38	17	-6.6	60.9	Mean	7.35	26	14	-9.8	79.5
SE	0.04	3		1.5	11.4	SE	0.11	12	2	2.9	20.2	SE	0.05	4	1	1.7	10.0
p*		.02				p*			.05		.005	p*		.01	.005	.005	.01

*p = paired statistical analysis

TABLE VI. FLUID VOLUME ADMINISTERED AND CHANGES IN HEMATOCRIT IN BABOONS SUBJECTED TO ESCHERICHIA COLI SHOCK

Group	Description of group	Baboon No.	Saline Infusion Volume, ml/kgm	Saline Infusion Rate, ml/kgm/hr	Hematocrit Initial	Hematocrit Final	Final observation time, hrs
A	E. coli alone	1	41	4.1	49	49	10
		4	16	5.3	46	46	3
		7	20	4.0	49	52	5
		10	97	8.1	36	38	12
		13	57	4.8	45	54	12
		Mean	46	5.3	45	48	8
		SE	15	0.7	2	3	2
		p*				NS	
B	E. coli + methylprednisolone + gentamicin	2	118	4.9	49	49	24
		6	70	2.9	42	39	24
		8	62	5.2	43	40	12
		11	62	5.2	44	38	12
		14	41	3.4	45	44	12
		Mean	71	4.3	45	42	17
		SE	13	0.5	1	2	3
		p*				NS	
		p†		NS			
C	E. coli + gentamicin	3	62	2.6	43	39	24
		9	78	6.5	42	42	12
		12	73	6.1	40	40	12
		15	70	5.8	44	40	12
		Mean	71	5.3	42	40	15
		SE	3	0.9	1	1	3
		p*				NS	
		p§		NS			

*paired analysis

†unpaired analysis, A to B

§unpaired analysis, A to C

TABLE VII. FIBRINOGEN DEGRADATION PRODUCT CONCENTRATIONS IN BABOONS SUBJECTED TO LD_{100} ESCHERICHIA COLI SHOCK (μg/ml)

Group	Description of group	Baboon No.	Control	Hours						Days
				2	3	4	8	12	24	7-28
A	E. coli alone	1	<10	>40<120		>240<320	>400<480			
		4	<10	>160<200	>360<400					
		7	<10	>80<120		>320<360				
		10	<10	>10< 40		>80<120	>40< 80	>40< 80		
		13	<10	>120<160		>120<160	>80<120	>80<120		
B	E. coli + methylprednisolone + gentamicin	2	<10	>10< 40		>120<160	>360<420	>360<420		>40< 60
		6	<10	>80<120		>120<160	>320<360	>320<360	>160<200	>10< 40
		8	<10	>40< 80		>160<200	>440<480	>480<520		>10< 40
		11	>160<200	>40< 80		>40< 80	>10< 40	>40< 80		--
		14	<10	>10< 40		>80<120	>240<280	>240<280		
C	E. coli + gentamicin	3	<10	>10< 40		>80<120	>120<160	>120<160	>160<200	
		9	<10	>40< 80		>120<160	>160<200	>80<120		
		12	<10	>320<360		>440<480	>200<240	>200<240		
		15	<10	>80<120		>160<200	>160<200	>80<120		

TABLE VIII. ESCHERICHIA COLI BLOOD CONCENTRATIONS IN BABOONS SUBJECTED TO LD_{100} ESCHERICHIA COLI SHOCK AND TREATED WITH STEROID AND ANTIBIOTIC

Experimental group*		Mean no. org. infused per kgm. body wt.	Zero time	+29 min	+125 min	+4 hr	+6 hr	+12 hr
A	Mean	$2.1X10^{10}$	Negative	$1.3X10^{7}$	$1.8X10^{7}$	$2.9X10^{2}$	$1.7X10^{2}$	$1.6X10^{3}$
	SE	$(0.1X10^{10})$		$(0.2X10^{7})$	$(0.7X10^{7})$	$(1.1X10^{2})$	$(0.7X10^{2})$	$(0.7X10^{3}$
	N†	5	5	5	5	4	4	2
	p§							0.05
B	Mean	$2.6X10^{10}$	Negative	$1.3X10^{7}$	$1.9X10^{7}$	$4.8X10^{2}$	$2.3X10^{2}$	$2.3X10^{2}$
	SE	$(0.4X10^{10})$		$(0.2X10^{7})$	$(0.2X10^{7})$	$(1.4X10^{2})$	$(0.5X10^{2})$	$(0.7X10^{2}$
	N	5	5	5	4	4	5	4
	pΦ						0.02	
C	Mean	$3.0X10^{10}$	Negative	$2.1X10^{7}$	$2.1X10^{7}$	$3.7X10^{3}$	$6.0X10^{1}$	$2.9X10^{2}$
	SE	$(0.6X10^{10})$		$(0.5X10^{7})$	$(0.6X10^{7})$	$(3.5X10^{3})$	(0)	$(1.5X10^{2}$
	N	4	4	4	4	4	2	3

*A, Escherichia coli alone;
B, Escherichia coli + methylprednisolone sodium succinate + gentamicin sulfate;
C, Escherichia coli + gentamicin sulfate.

†Number of baboons.

§Unpaired analysis (A vs. B).

ΦUnpaired analysis (B vs. C).

TABLE IX. Serum bacteriostatic, bactericidal and gentamicin sulfate levels[a] in baboons subjected to LD_{100} Escherichia coli shock

Experimental Group[b]		Zero time	[N][c]	+3-5 hr	[N]	+8.5 hr	[N]	+12 hr	[N]
		Serum Bacteriostatic (dilution)							
A	Mean	1:3.3 (1.6)	[4]	1:2	[1]	--		--	
	Range	1:1 to 1:8		--		--		--	
B	Mean	1:1.2 (0.4)	[6]	1:54.4 (20)	[5]	1:23.2 (5.7)	[5]	1:16 (0)	[4]
	Range	0 to 1:4		1:16 to 1:128		1:4 to 1:32			
C	Mean	1:1.8 (0.8)	[4]	1:32 (11.3)	[4]	1:30 (12.4)	[4]	1:69.3 (32.4)	[3]
	Range	1:1 to 1:4		1:16 to 1:64		1:8 to 1:64		1:16 to 1:128	
		Serum Bactericidal (dilution)							
A	Mean	1:2.5 (1.9)	[4]	1:1	[1]	--		--	
	Range	0 to 1:8							
B	Mean	1:0.8 (0.4)	[6]	1:44 (12)	[4]	1:28 (4.0)	[4]	1:16 (0)	[4]
	Range	0 to 1:4		1:16 to 1:64		1:16 to 1:32		--	
C	Mean	1:2.0 (2.0)	[3]	1:40 (24)	[2]	1:40 (24)	[2]	1:40 (24)	[2]
	Range	0 to 1:4		1:16 to 1:64		1:16 to 1:64		1:16 to 1:64	
		Serum Gentamicin (µg/ml							
A	Mean	--		--		--		--	
	Range	--							
B	Mean	--		16.9 (1.9)	[5]	8.2 (1.3)	[5]	8.2[d] (1.2)	[4]
	Range	--		10 to 21		3 to 10.3		6 to 10.5	
C	Mean	--		18.9 (2.3)	[4]	14.7 (3.5)	[4]	21.0 (2.6)	[3]
	Range	--		15 to 25		5.3 to 22		17 to 26	

[a] Mean(±SE) of measured values

[b] Group designations: A, Escherichia coli alone; B, Escherichia coli + methylprednisolone succinate + Gentamicin sulfate; C, Escherichia coli + gentamicin sulfate

[c] [Number of observations]

[d] =< 0.05 Group B compared to Group C

LEGENDS FOR FIGURES

Figure 1. Effects of LD_{100} Escherichia coli (E. coli) infusion on mean arterial blood pressure and heart rate in baboons (mean±SE).

Group A--E. coli alone

Group B--E. coli plus methylprednisolone sodium succinate (MPSS) and gentamicin sulfate (GS)

Group C--E. coli plus GS

* = $p<0.05$, compared to zero time value

Figure 2. Changes in total white blood cell, mature and immature neutrophil concentrations and platelet concentrations following LD_{100} Escherichia coli (E. coli) infusions in baboons (mean±SE).

Group A--E. coli alone

Group B--E. coli plus methylprednisolone sodium succinate (MPSS) and gentamicin sulfate (GS)

Group C--E. coli plus GS

* = $p<0.05$, compared to zero time value

Δ = $p<0.05$, comparison of Groups A and B

† = $p<0.05$, comparison of Groups B and C

Figure 3. In vitro study with baboon serum showing effects of gentamicin sulfate (GS) on colony-forming units of Escherichia coli (E. coli). Effects of methylprednisolone sodium succinate (MPSS) and GS, separately and in combination with E. coli, are contrasted. Comparison with in vivo state is also shown.

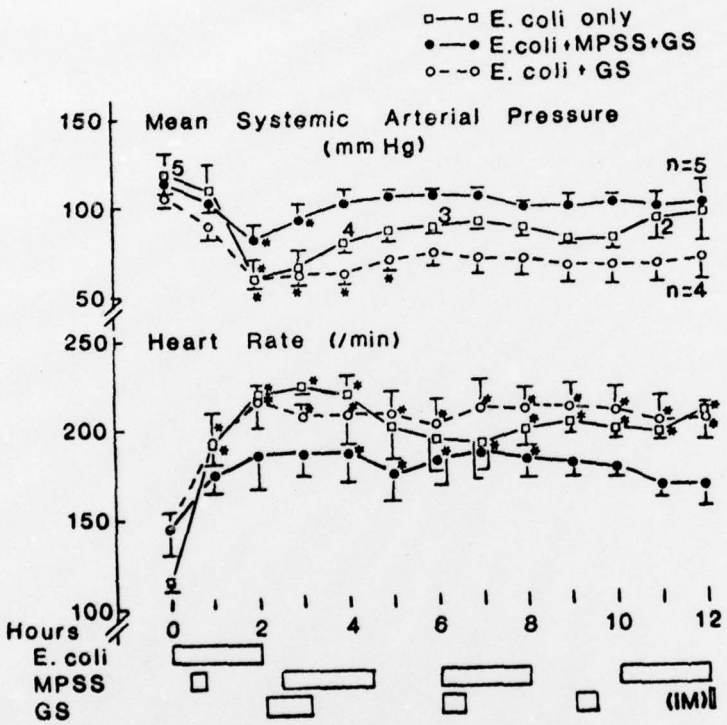
E. coli only
E.coli+MPSS+GS
E. coli + GS
Mean Systemic Arterial Pressure
(mm Hg)
150
100
50
n=5
n=4
Heart Rate (/min)
250
200
150
100
Hours
0
2
4
6
8
10
12
E. coli
MPSS
GS
(IM)

FIG. 1

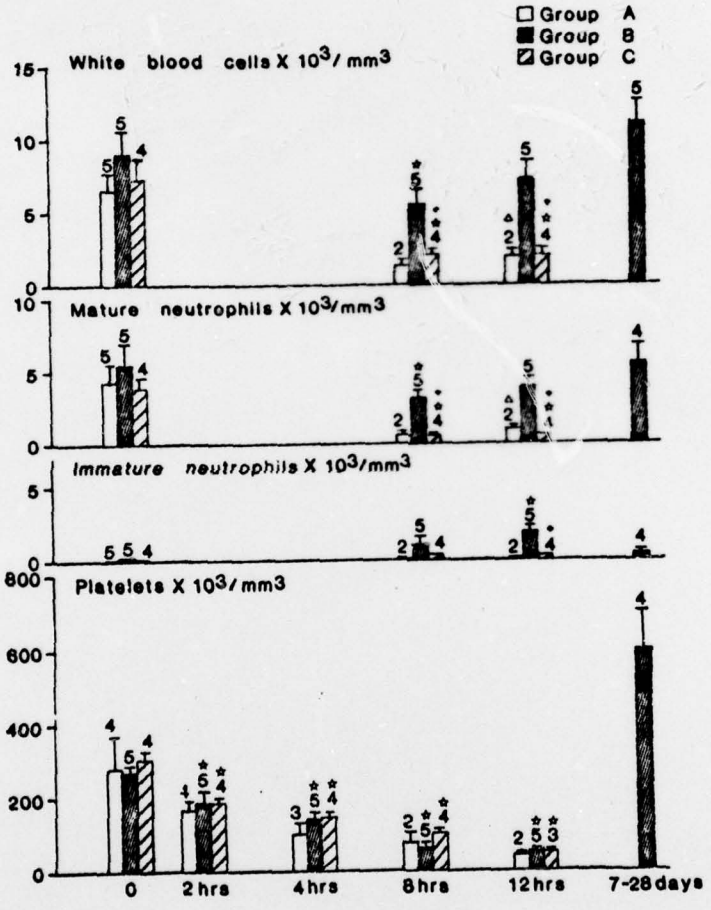
Group A
Group B
Group C
White blood cells X 10³/mm³
Mature neutrophils X 10³/mm³
Immature neutrophils X 10³/mm³
Platelets X 10³/mm³
0
2 hrs
4 hrs
8 hrs
12 hrs
7-28 days

FIG. 2

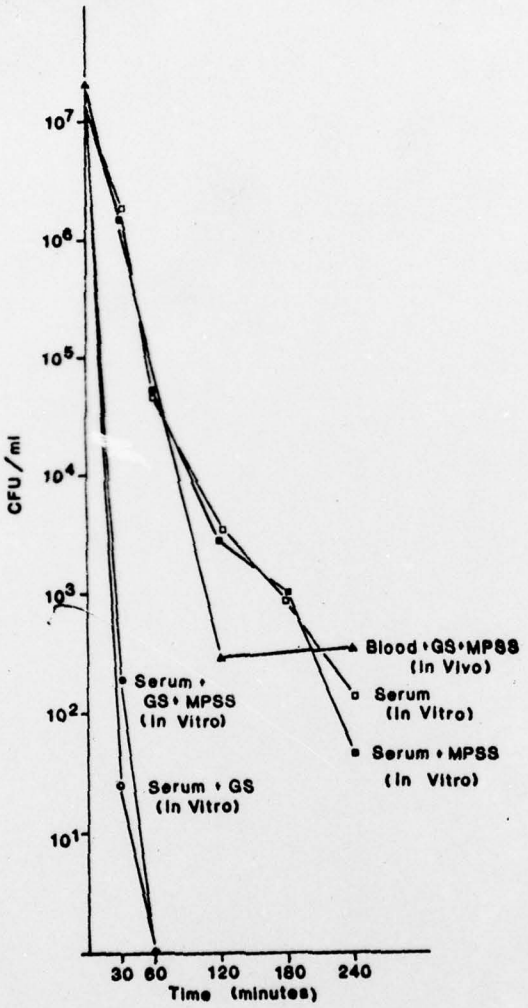
CFU / ml
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Blood + GS + MPSS (In Vivo)
Serum (In Vitro)
Serum + MPSS (In Vitro)
Serum + GS + MPSS (In Vitro)
Serum + GS (In Vitro)
30 60 120 180 240
Time (minutes)

FIG. 3

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Fourteen adult baboons (P. c. cynocephalus) were anesthetized and prepared aseptically for 2-hr infusions of 2.4×10^{10} E. coli organisms per kg body wt. Three groups were studied: Group I, E. coli alone; Group II, E. coli plus infusions of both gentamicin sulfate (GS)(18 mg/kg) and methylprednisolone sodium succinate (MPSS) (75 mg/kg); and Group III, E. coli plus GS (18 mg/kg). Animals were monitored during a 12-hr period and observed 7-21 days. Systemic hypotension occurred in all groups within 2 hr of E. coli infusion.

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Subsequent hypotension, hypoglycemia and death were prevented in all fully treated baboons (Group II) while all animals of Groups I and III died within 42 hr. Platelet and WBC concentrations declined in all animals ($p<0.05$) while WBC values recovered ($p<0.05$) in Group II. Lactic acid and pCO_2 values were inversely related in all animals, while pO_2 and pH remained relatively constant. In vivo blood concentrations of E. coli at 2-12 hr were relatively similar in all groups ($2.3x10^2$ to $1.5x10^3$ org/ml, 12 hr). In vitro studies utilizing GS with or without MPSS demonstrated the absence of organisms as early as 3 hr. Findings demonstrate the importance of maintenance infusions of both steroid and antibiotic in promoting survival in LD_{100} E. coli-induced shock.

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